

Further Chemical Analysis of Holothurin, the Saponin-like Steroid from the Sea-cucumber

J. D. CHANLEY, STELLA K. KOHN, ROSS F. NIGRELLI & HARRY SOBOTKA

*Department of Chemistry, The Mount Sinai Hospital, New York; and
New York Aquarium, New York Zoological Society*

IN continuation of our work (Nigrelli, et al., 1955) on the toxic principle of the sea-cucumber *Actinopyga agassizi*, we have found that the molecule contains a sulfuric acid group in ester linkage. Holothurin, after passing through a mixed bed ion exchange column, shows the following elementary composition: Found: C, 51.75; H, 8.00; S, 2.54; 2.86; acid equivalent, 1150. Calculated for $C_{50}H_{82}O_{26}S$: C, 52.2; H, 7.8; S, 2.78; acid equivalent, 1148.

That the sulfur is in the form of a sulfate is shown by the isolation of barium sulfate from the hydrochloric acid hydrolysate of the above material. Furthermore, hydrolysis without addition of acid by heating of the steambath with reflux for 54 hours, and subsequent removal of the precipitated aglycone, produces an increase of titrable acidity by 88 percent., showing the liberation of H_2SO_4 . The original neutral compound thus consists of the sodium salt of a sulfuric acid ester.

The aglycone, obtained by hydrolysis with methanolic hydrochloric acid, seems to be essentially uniform and corresponds to substance A (Nigrelli, et al., 1955), for which we pro-

pose the name *holothurinogen*. Its rotation is $[\alpha]_D^{25} = -17^\circ$ (in CCl_3H). Upon treatment with ether its melting point rises from 258° to ca. $274-276^\circ$, indicating the loss of some crystal methanol.

The infrared spectrum of *holothurinogen* shows bands at 3500 (hydroxyl), 1740 and 1080 (carbonyl), and 1635, 995 and 945 cm^{-1} (conjugated double bonds). The position of these functions in the molecule is under investigation.

SUMMARY

Holothurin is found to contain a sulfuric acid group in ester linkage. The infrared spectrum of the aglycone indicates the presence of a carbonyl group and a conjugated system of two double bonds. The name *holothurinogen* is proposed for the principle aglycone.

REFERENCE

- NIGRELLI, ROSS F., J. D. CHANLEY, STELLA K. KOHN & HARRY SOBOTKA
1955. The Chemical Nature of Holothurin, a Toxic Principle from the Sea-Cucumber (Echinodermata: Holothurioidea). *Zoologica*, 40: 47-48.